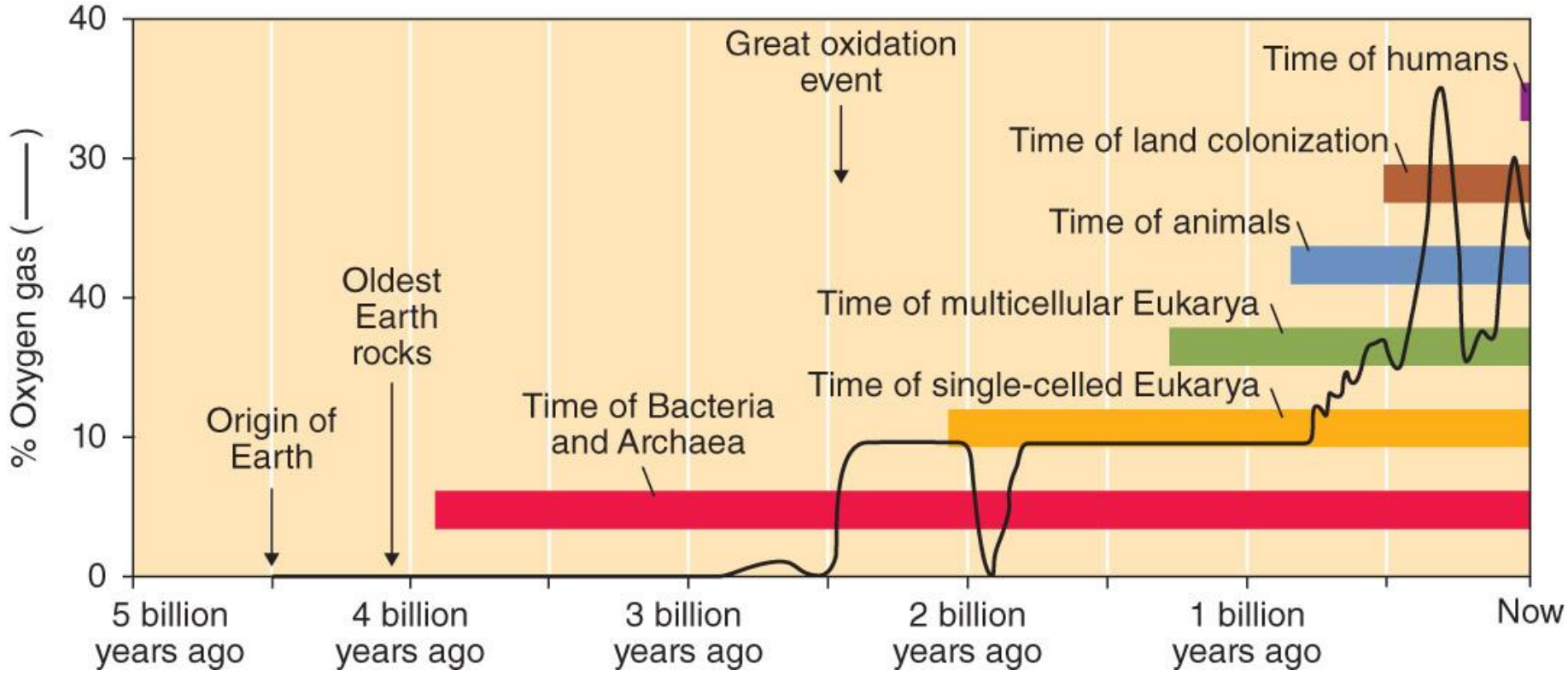
Microbial Genetics

- Geologic time
  - Fossil evidence of early prokaryotes 3.8 billion years ago
  - *Archaea* domains
  - Microbial evolution

Figure 7.1: Fossil Microbes

Courtesy of Abigail Allwood, Geologist at NASA Jet Propulsion Laboratory

The Appearance of Life on Earth



The Hereditary Molecule in All Organisms Is DNA

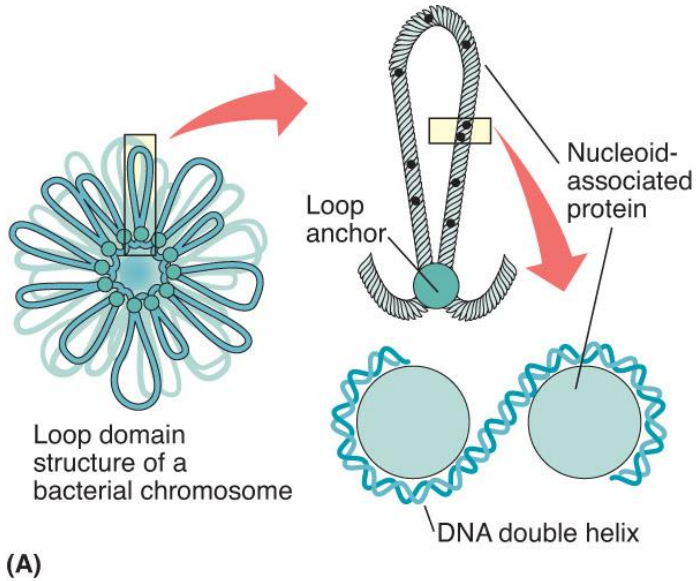
- Deciphering the structure of DNA
  - Rosalind Franklin
  - James Watson working with Francis Crick
  - Figured out that DNA is a double helix structure

Rosalind Franklin

- Bacterial and Archaeal DNA Is Organized within the Nucleoid
  - The DNA usually exists as a single, circular chromosome.

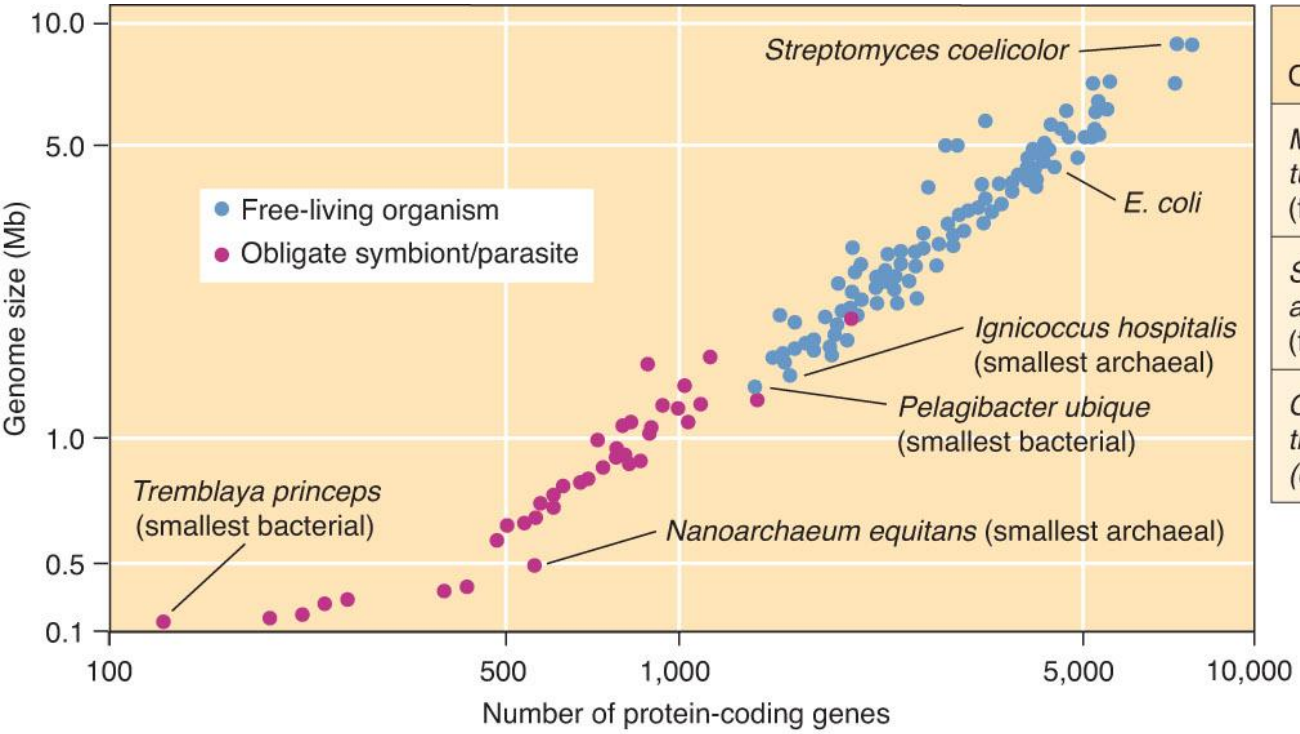
- DNA Within a Nucleoid is Highly Compacted

- Many Microbial Cells Also Contain Plasmids
  - Plasmids carry nonessential but often useful information.



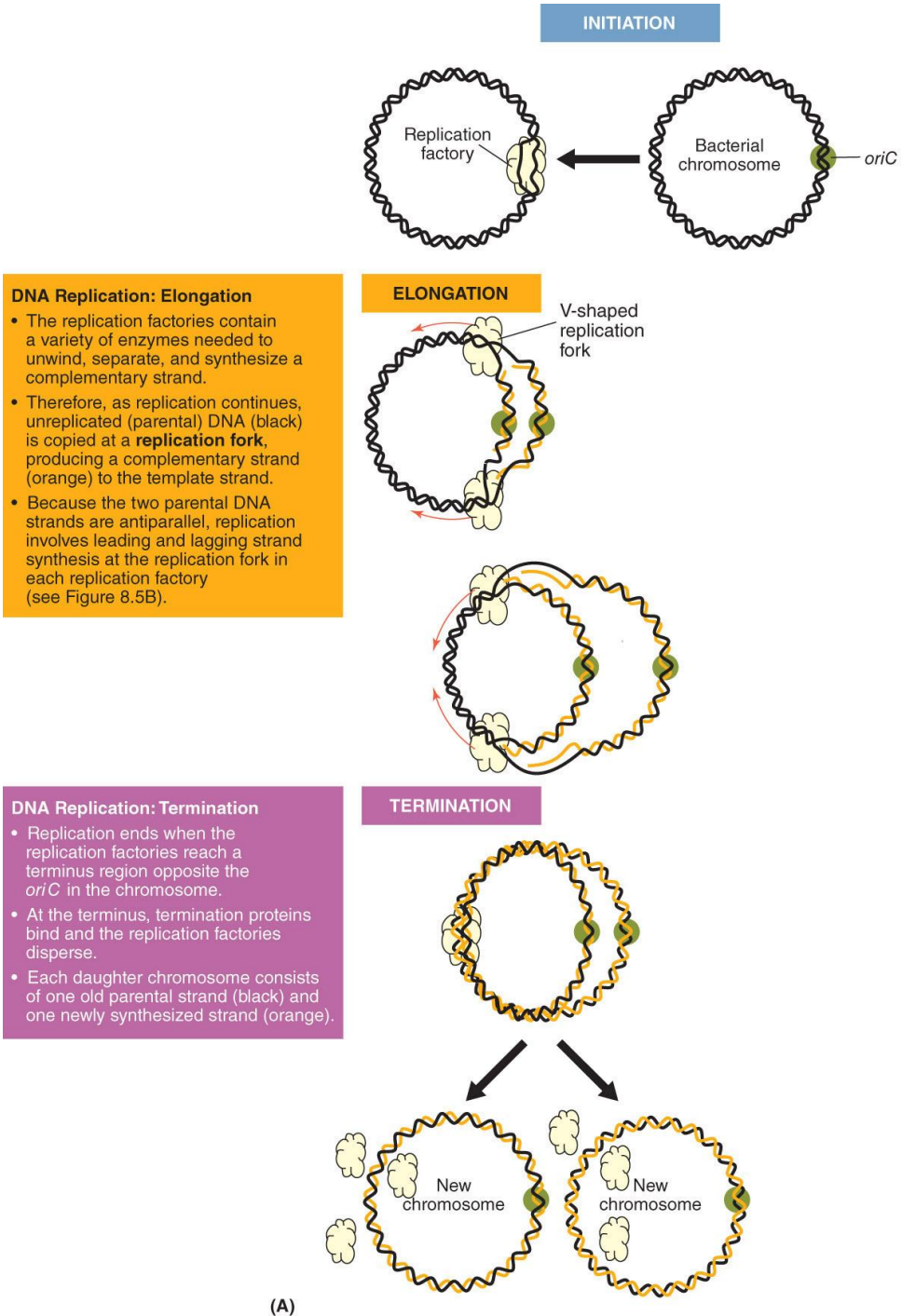
DNA Packing

Bacterial Genome (complete set of genes) Varies



Organism	Genome size (Mb)	Protein-coding genes
Mycobacterium tuberculosis (free-living)	4.4	3,900
Staphylococcus aureus (free-living)	2.8	2,700
Chlamydia trachomatis (obligate/parasite)	1.0	936

Genome Size Among the Bacteria and Archaea


INITIATION
Replication factory
Bacterial chromosome
oriC
DNA Replication: Elongation
• The replication factories contain a variety of enzymes needed to unwind, separate, and synthesize a complementary strand.
• Therefore, as replication continues, unreplicated (parental) DNA (black) is copied at a replication fork, producing a complementary strand (orange) to the template strand.
• Because the two parental DNA strands are antiparallel, replication involves leading and lagging strand synthesis at the replication fork in each replication factory (see Figure 8.5B).
ELONGATION
V-shaped replication fork
DNA Replication: Termination
• Replication ends when the replication factories reach a terminus region opposite the oriC in the chromosome.
• At the terminus, termination proteins bind and the replication factories disperse.
• Each daughter chromosome consists of one old parental strand (black) and one newly synthesized strand (orange).
TERMINATION
New chromosome
New chromosome
(A)

DNA Replication Occurs Before a Cell Divides

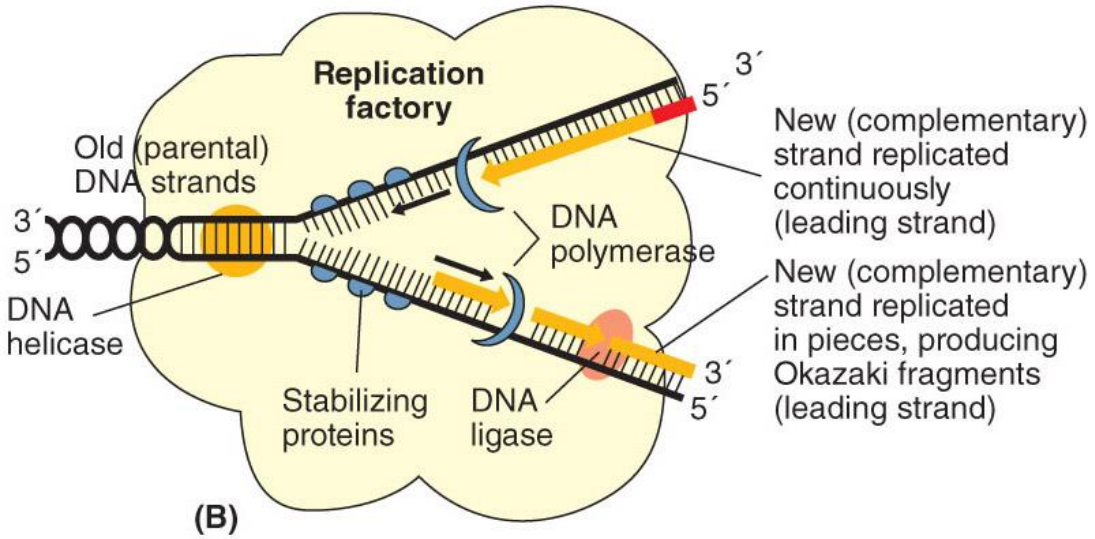
- Highly regulated process:
  - Initiation
  - Elongation
  - Termination

eplication of Circular Plasmid of *Escherichia coli*

- DNA Polymerase Only Reads in the 3' to 5' Direction
  - Leading strand synthesis
  - Lagging strand synthesis

DNA Replication: Initiation

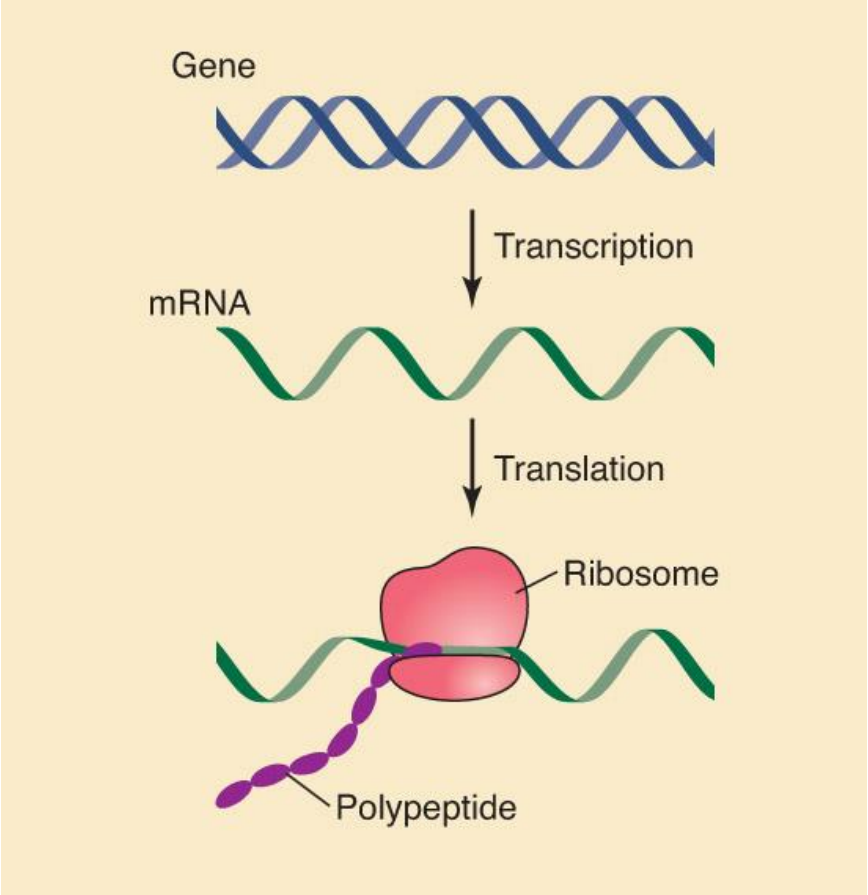
- Replication of the circular bacterial chromosome begins at a fixed region on the DNA called ***oriC***.
- At *oriC*, copies of initiation proteins bind to the DNA.
- Other proteins needed for replication will then add to this so-called "**replication factory.**"



Replication of Circular Chromosome of *Escherichia coli*

Gene Expression Produces RNA and Protein for Cell Function

- Central dogma identifies the flow of genetic material (DNA→ RNA→ protein).
- Transcription copies genetic information into complementary RNA.



The Central Dogma

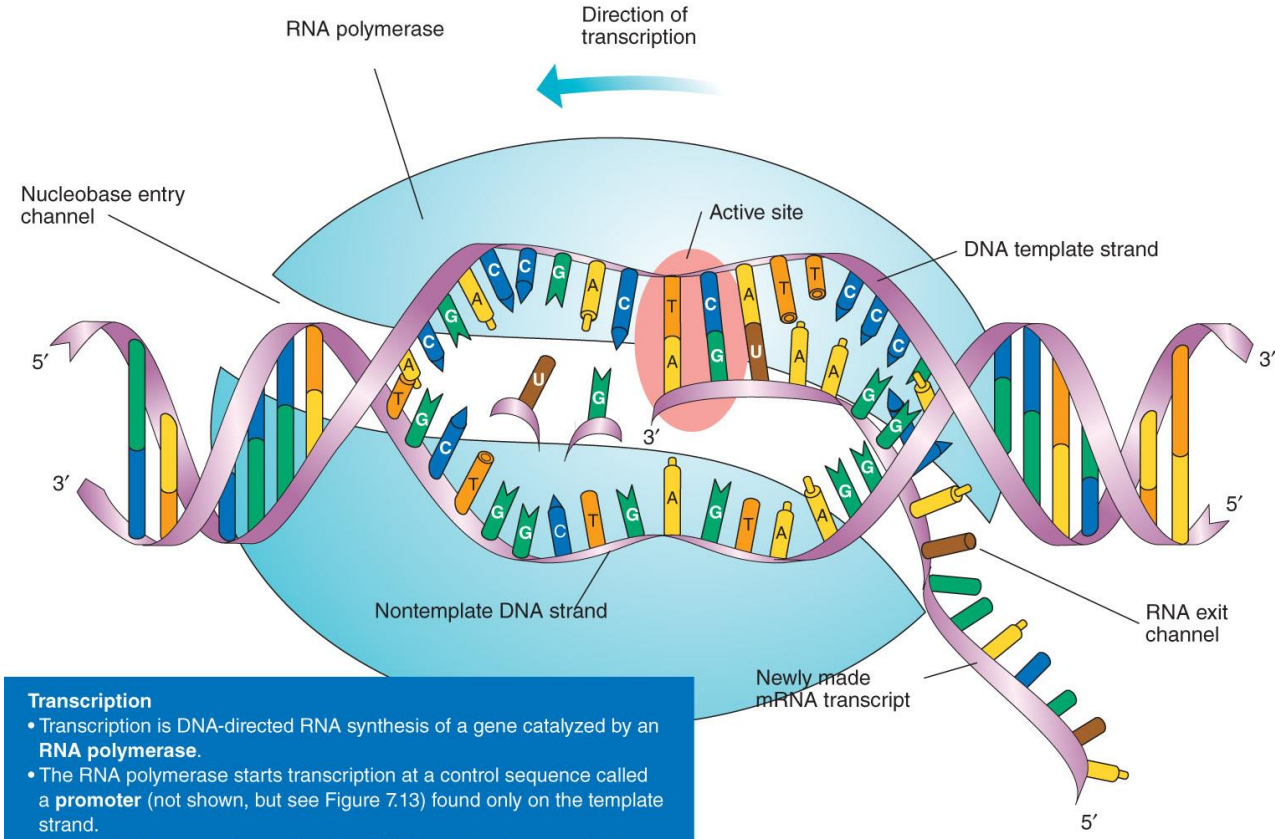
A Comparison of DNA and RNA

TABLE 7.2 A Comparison of DNA and RNA

DNA (Deoxyribonucleic Acid)	RNA (Ribonucleic Acid)
In Bacteria and Archaea, found in the nucleoid and plasmids; in Eukarya, found in the nucleus, mitochondria, and chloroplasts	In all organisms, found in the cytosol and in ribosomes; in Eukarya, also found in the nucleolus
Always associated with chromosome (genes); each chromosome has a fixed amount of DNA	Found mainly in combinations with proteins in ribosomes (ribosomal RNA), as messenger RNA, and as transfer RNA
Contains a 5-carbon sugar called deoxyribose	Contains a 5-carbon sugar called ribose
Contains bases adenine, guanine, cytosine, and thymine	Contains bases adenine, guanine, cytosine, and uracil
Contains phosphorus (in phosphate groups) that connects deoxyribose sugars with one another	Contains phosphorus (in phosphate groups) that connects ribose sugars with one another
Functions as the molecule of inheritance	Functions in protein synthesis and gene regulation
Large double-stranded molecule	Small single-stranded molecule

Comparison of DNA and RNA

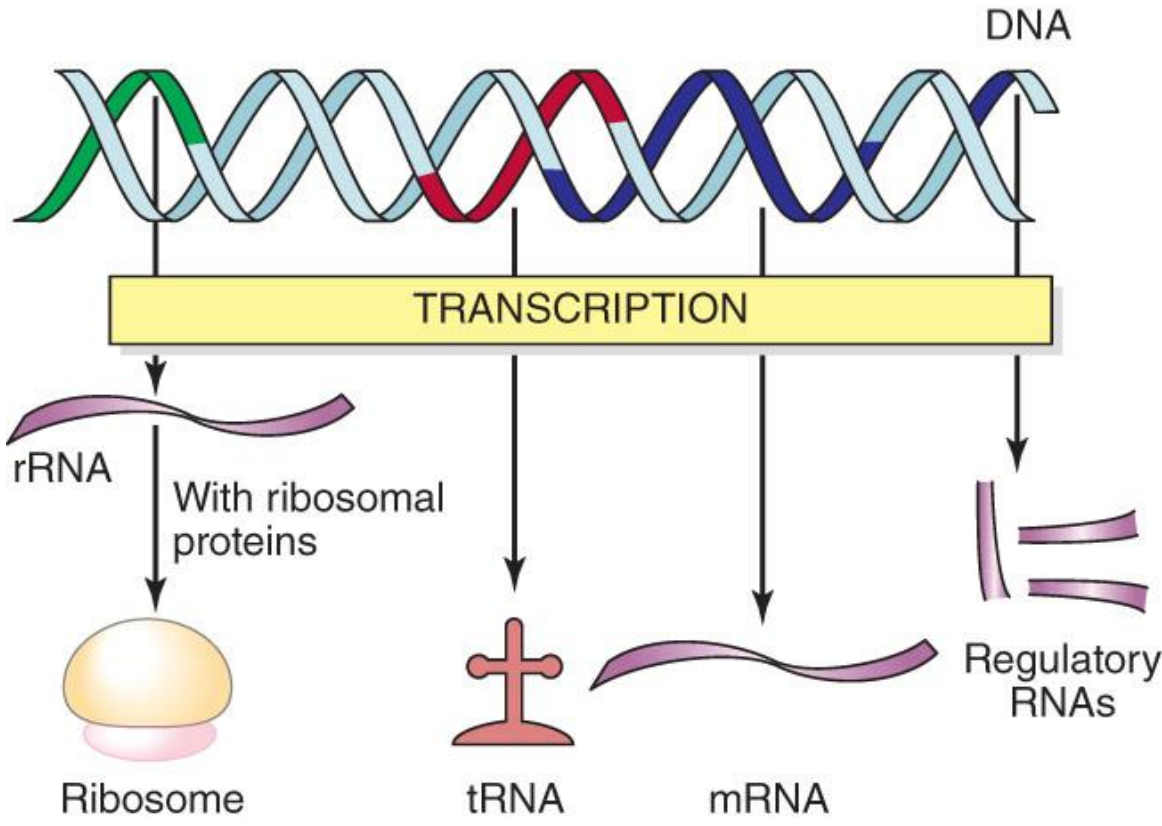
- Transcription Copies Genetic Information into Complementary RNA
  - RNA polymerase reads the DNA template
  - Promoter starts transcription
  - Terminator ends it



Transcription Process During Elongation

Transcription
- Transcription is DNA-directed RNA synthesis of a gene catalyzed by an **RNA polymerase**.
- The RNA polymerase starts transcription at a control sequence called a **promoter** (not shown, but see Figure 7.13) found only on the template strand.
- The RNA polymerase transcribes the DNA template strand, substituting uracil for thymine where adenine appears in the DNA template strand.
- In Bacteria and Archaea, transcription stops at DNA sequences called **terminators** (not shown). The resulting transcript is released as a single polynucleotide strand.

- Types of RNA:
  - mRNA carries codon to ribosome
  - rRNA together with proteins make up ribosomes
  - tRNA carries anticodon and amino acid to ribosome

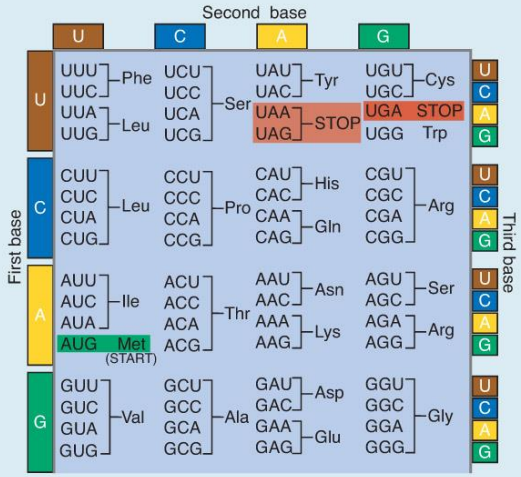


Transcription of the Three Types of RNA

- The Genetic Code Consists of Three-Letter Words

TABLE 7.3 The Genetic Code Decoder

The genetic code embedded in an mRNA is decoded by knowing which codon specifies which amino acid. On the far left column, find the first letter of the codon; then find the second letter from the top row; finally read up or down from the right-most column to find the third letter. The three-letter abbreviations for the amino acids are given. Note: In the Bacteria, AUG codes for formylmethionine (fMet) when starting a polypeptide.



Key

Ala = Alanine
Arg = Arginine
Asn = Asparagine
Asp = Aspartic acid
Cys = Cysteine
Gln = Glutamine
Glu = Glutamic acid
Gly = Glycine
His = Histidine
Ile = Isoleucine
Leu = Leucine
Lys = Lysine
Met = Methionine
Phe = Phenylalanine
Pro = Proline
Ser = Serine
Thr = Threonine
Trp = Tryptophan
Tyr = Tyrosine
Val = Valine

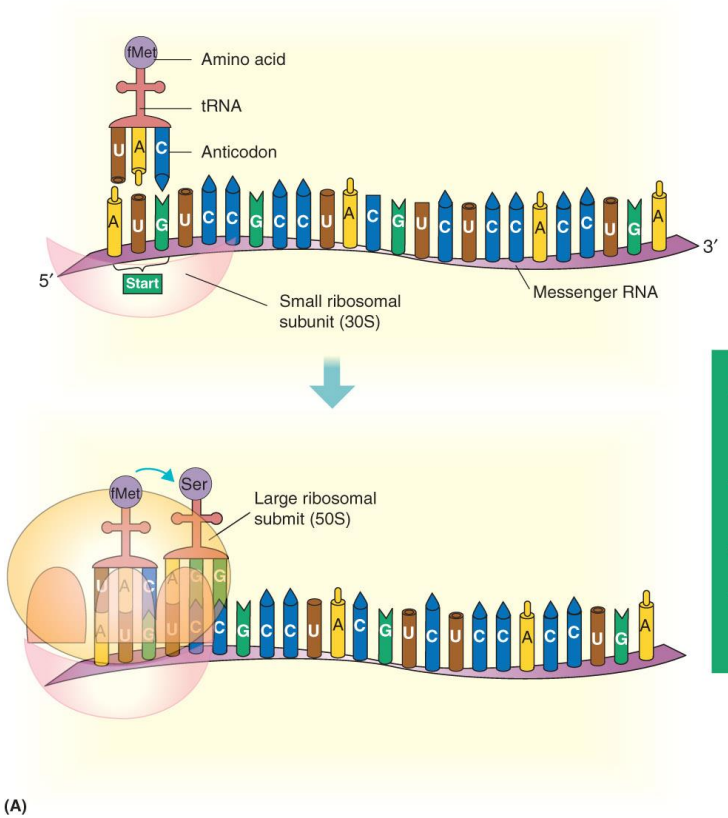
Genetic Code Decoder

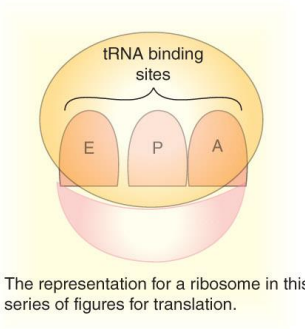
- Each sequence is made up of “three-letter words” called codons

Code is redundant, more than one codon specifies a specific amino acid

- Translation is the process of making the polypeptide at the ribosome.

  - Chain initiation
  - Chain elongation
  - Chain termination/ release





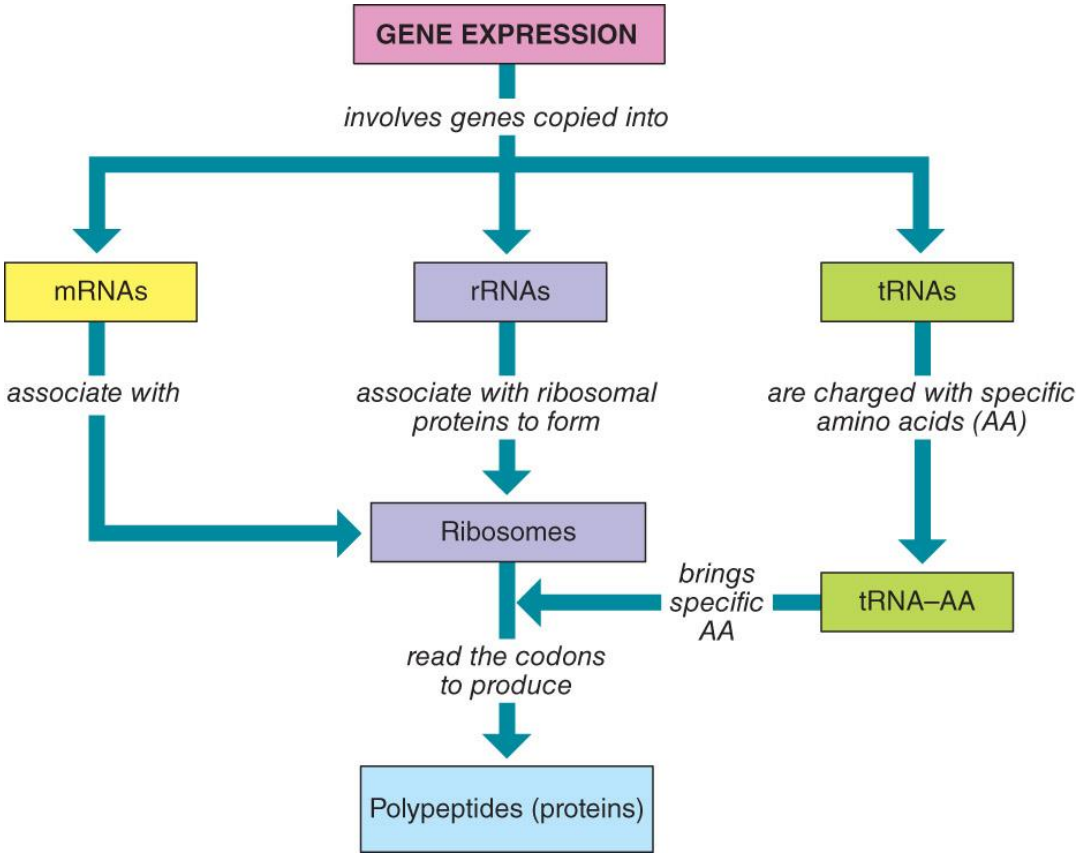
The representation for a ribosome in this series of figures for translation.

(A) Translation: Chain Initiation

- Translation starts when the translation components [small ribosomal subunit, initiator tRNA, and other protein factors (not shown)] assemble on the start codon.
- The large ribosomal subunit then associates to form a functional ribosome with three binding sites, called A, P, and E.
- After the initiator tRNA moves to the P site, the next tRNA can bind to the A site.
- A ribozyme of the large subunit then transfers the amino acid from the first tRNA onto the amino acid on tRNA in the A site.

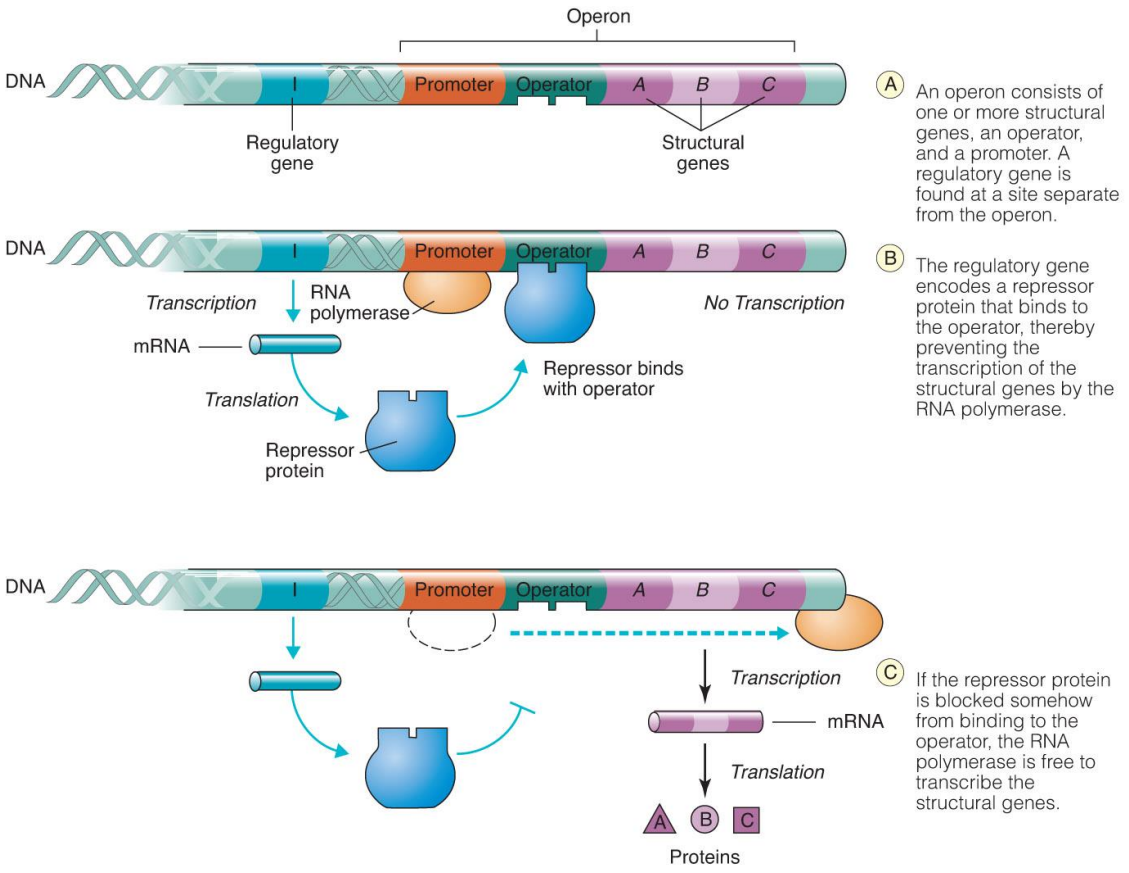
Protein Synthesis in a Bacterial Cell

- **Antibiotics Interfere with Gene Expression**



Concept Map for Protein Synthesis

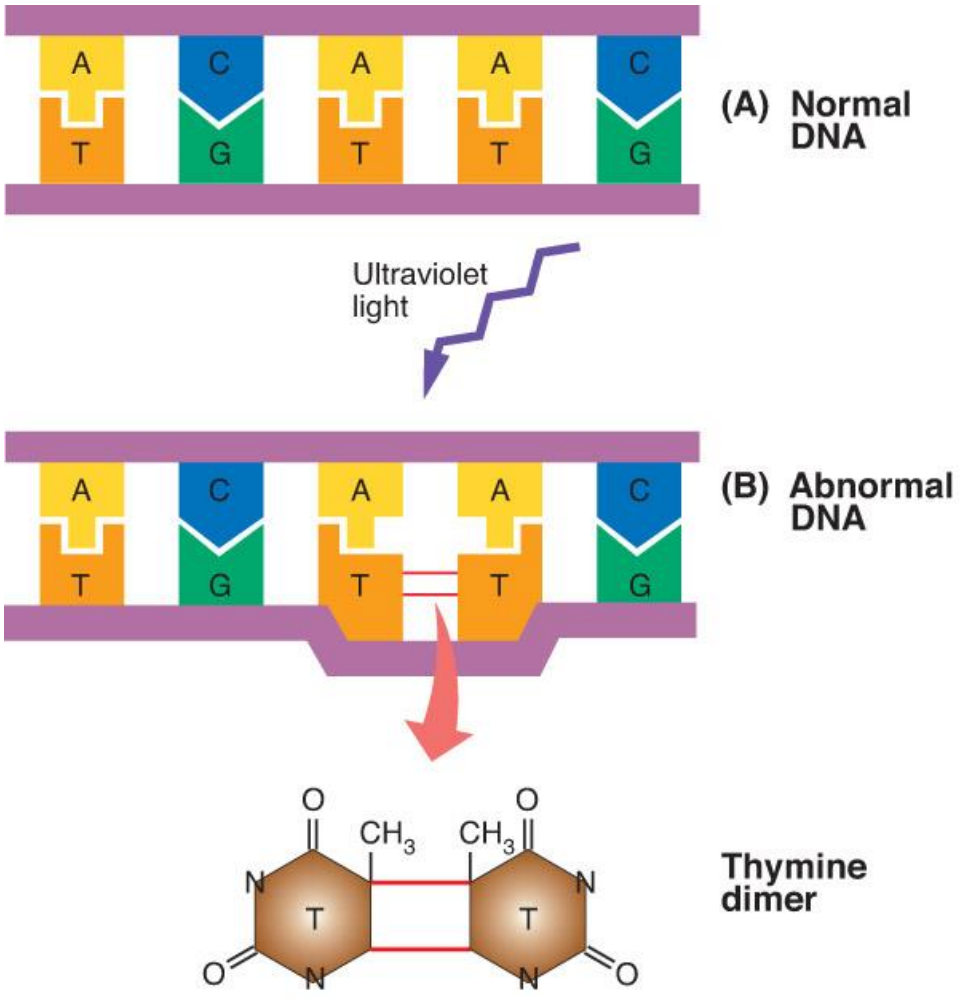
- Gene expression can be controlled in several ways.
  - An operon consists of a regulatory gene, promoter, structural genes, and a repressor.
  - Negative control, too much product serves as a repressor and turns off the gene.



Operon and Negative Control

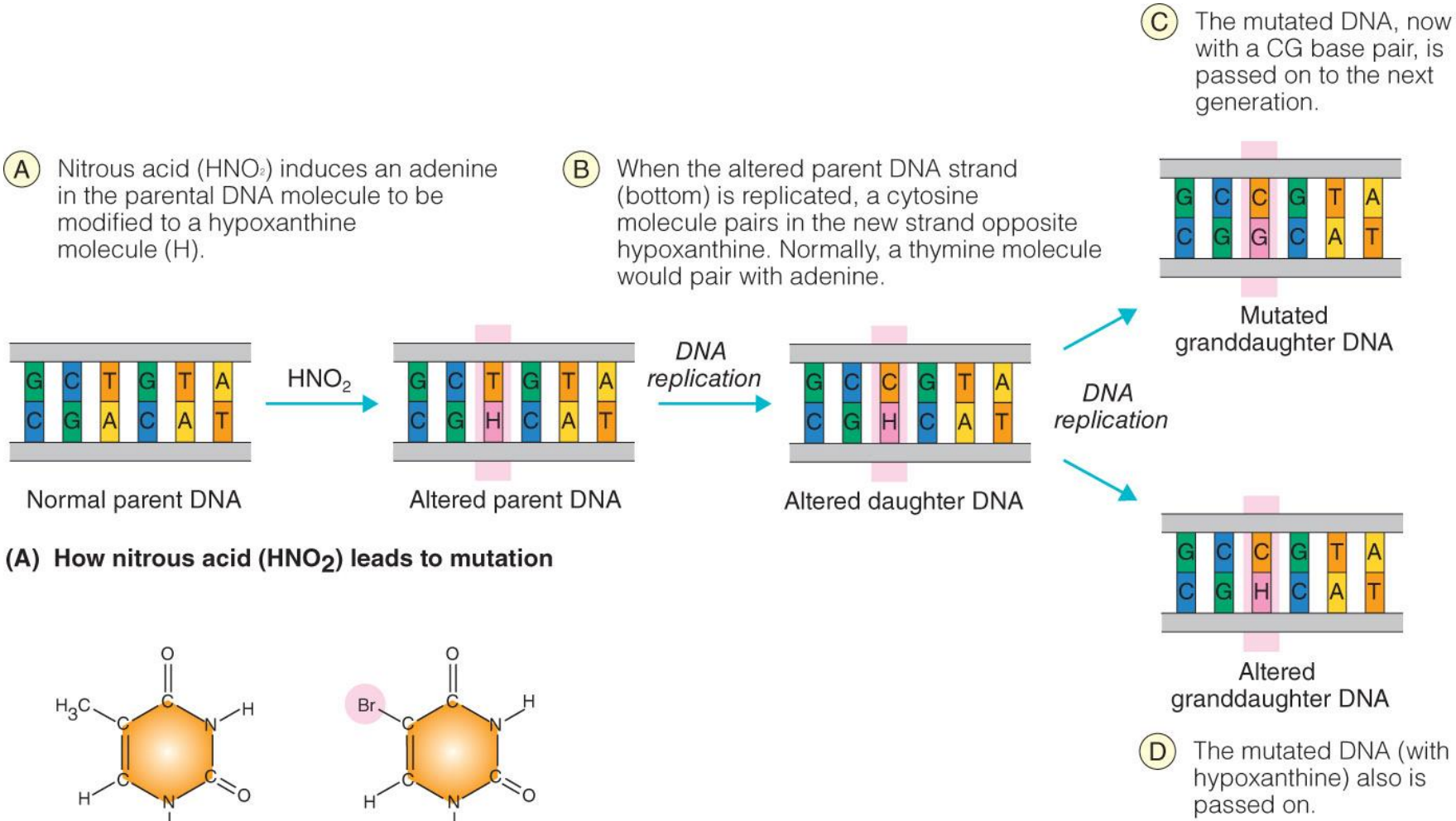
Mutations Are Permanent Changes in a Cell's DNA

- Mutations are the result of heritable changes in a genome
- Mutations can be spontaneous or induced.
  - Physical mutations
  - Chemical mutagens



Ultraviolet Light and DNA

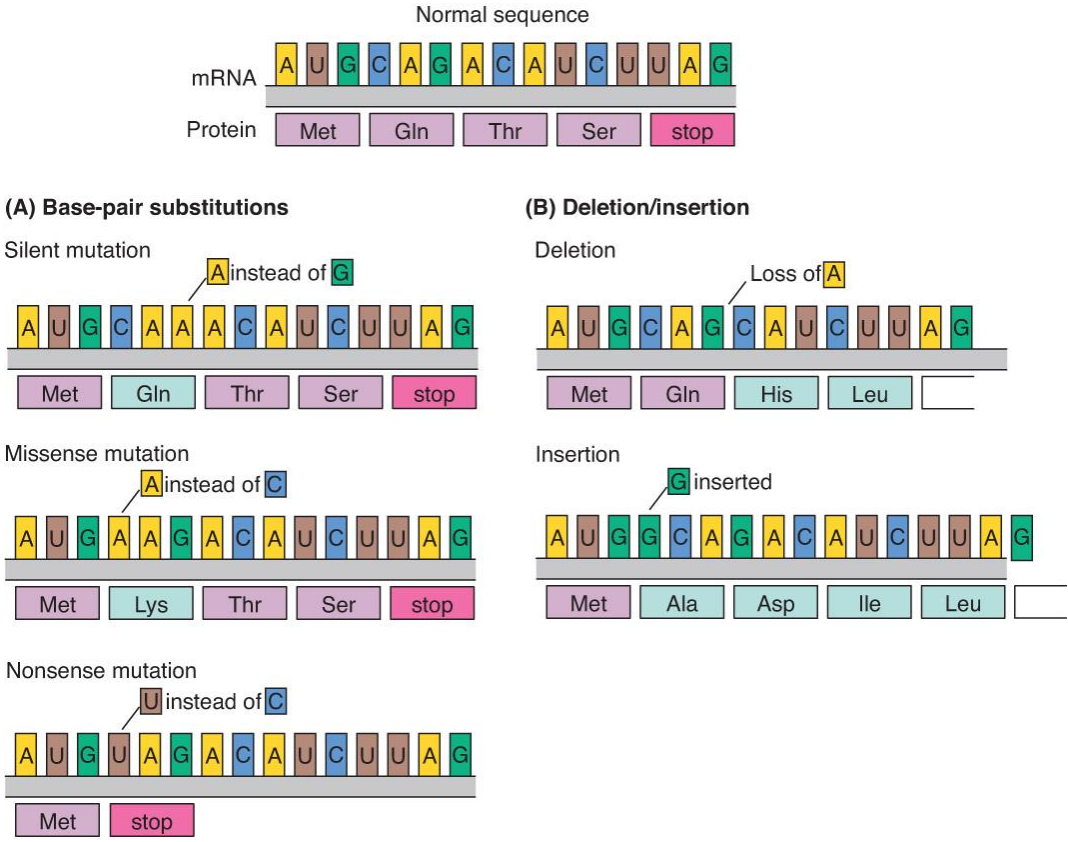
The Effect of Chemical Mutagens


A Nitrous acid (HNO_2) induces an adenine in the parental DNA molecule to be modified to a hypoxanthine molecule (H).
B When the altered parent DNA strand (bottom) is replicated, a cytosine molecule pairs in the new strand opposite hypoxanthine. Normally, a thymine molecule would pair with adenine.
C The mutated DNA, now with a CG base pair, is passed on to the next generation.
HNO_2
DNA replication
DNA replication
Normal parent DNA
Altered parent DNA
Altered daughter DNA
Mutated granddaughter DNA
Altered granddaughter DNA
D The mutated DNA (with hypoxanthine) also is passed on.
(A) How nitrous acid (HNO_2) leads to mutation
Thymine
5-bromouracil
(B) Thymine and its mutation-causing base analog

Effect of Chemical Mutagens

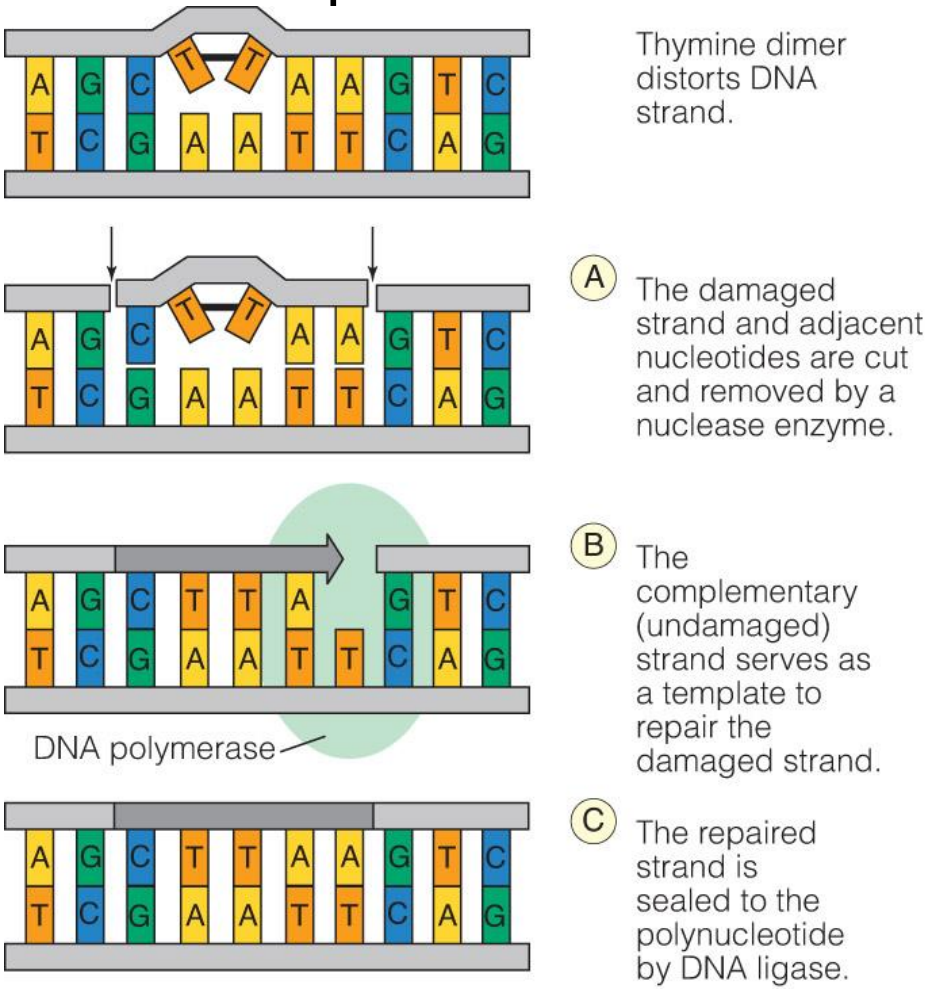
• Point Mutations Can Affect Gene Expression

- Point mutations can affect protein structure and function
- Base-pair substitutions
  - Silent mutation
  - Missense mutation
  - Nonsense mutation
- Base-pair deletion or insertion


Normal sequence
mRNA
A U G C A G A C A U C U U A G
Protein
Met Gln Thr Ser stop
(A) Base-pair substitutions
(B) Deletion/insertion
Silent mutation
A instead of G
A U G C A A A C A U C U U A G
Met Gln Thr Ser stop
Deletion
Loss of A
A U G C A G C A U C U U A G
Met Gln His Leu
Missense mutation
A instead of C
A U G A A G A C A U C U U A G
Met Lys Thr Ser stop
Insertion
G inserted
A U G G C A G A C A U C U U A G
Met Ala Asp Ile Leu
Nonsense mutation
U instead of C
A U G U A G A C A U C U U A G
Met stop

Categories and Results of Point Mutations

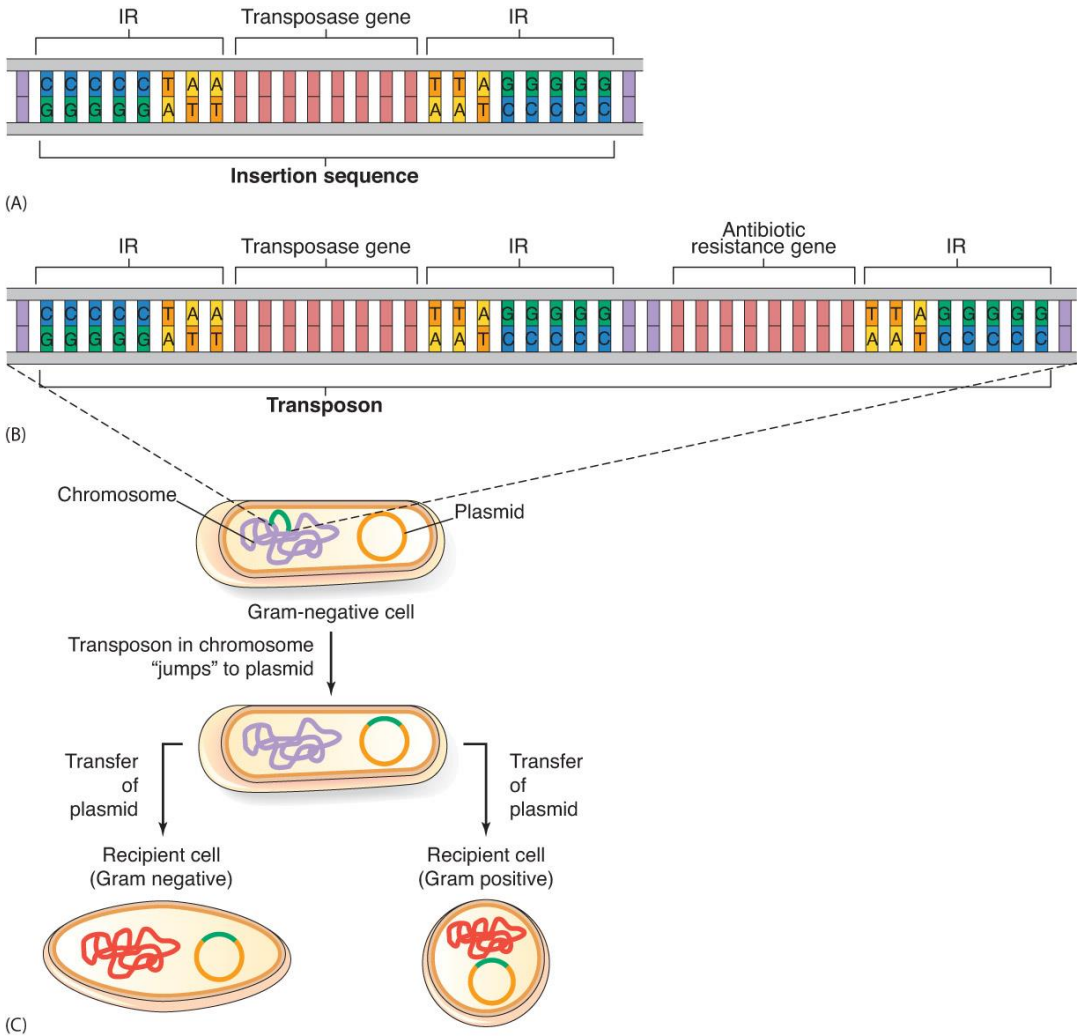
- Repair Mechanisms Attempt to Correct Mistakes or Damage in the DNA
  - Mismatch repair
  - Nucleotide excision repair


Thymine dimer distorts DNA strand.
A
The damaged strand and adjacent nucleotides are cut and removed by a nuclease enzyme.
B
The complementary (undamaged) strand serves as a template to repair the damaged strand.
DNA polymerase
C
The repaired strand is sealed to the polynucleotide by DNA ligase.

Nucleotide Excision Repair

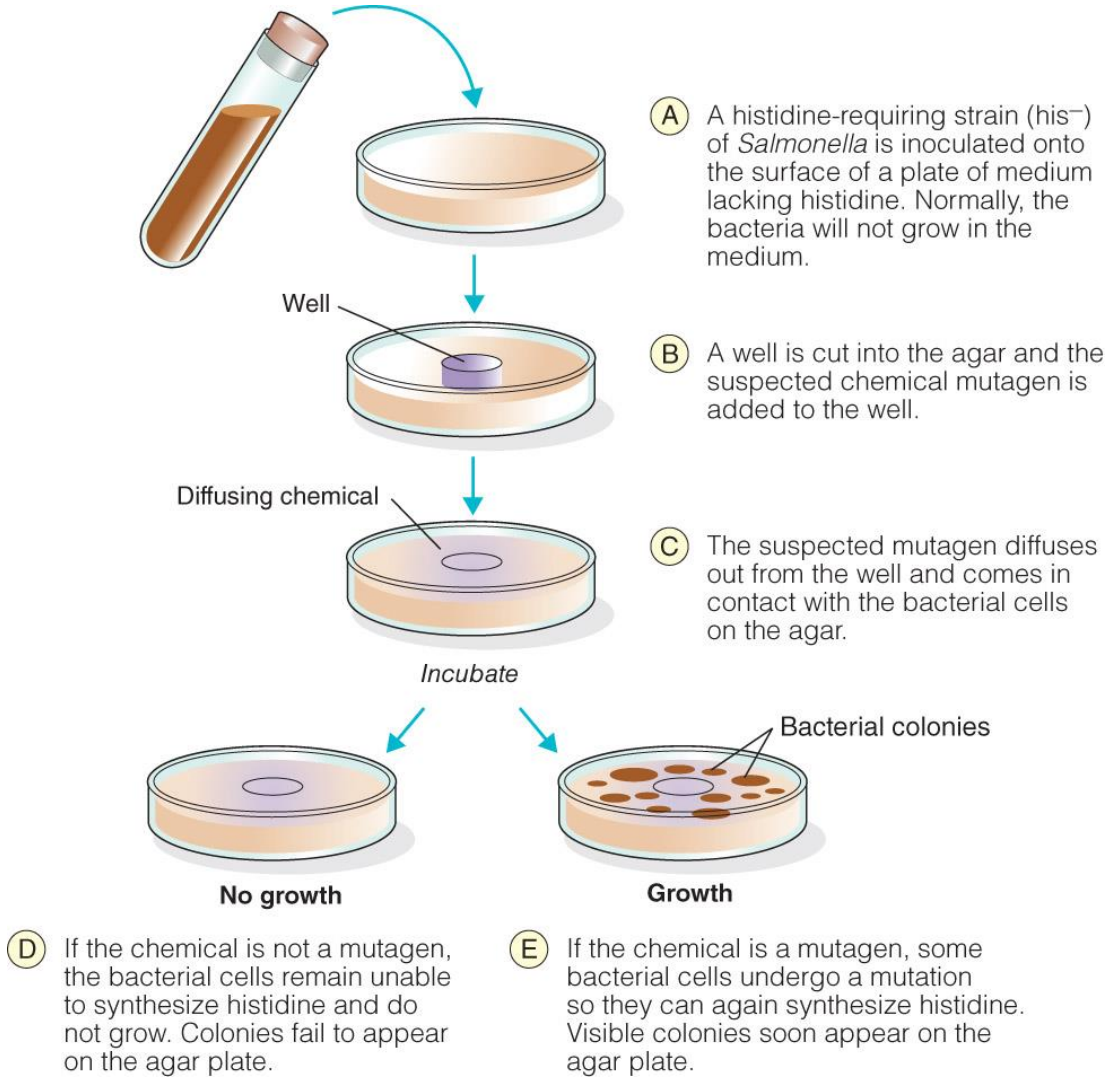
- Transposable genetic elements can cause mutations.
  - Referred to as “jumping genes” for their ability to move from one DNA site and integrate into another site

Transposable Elements



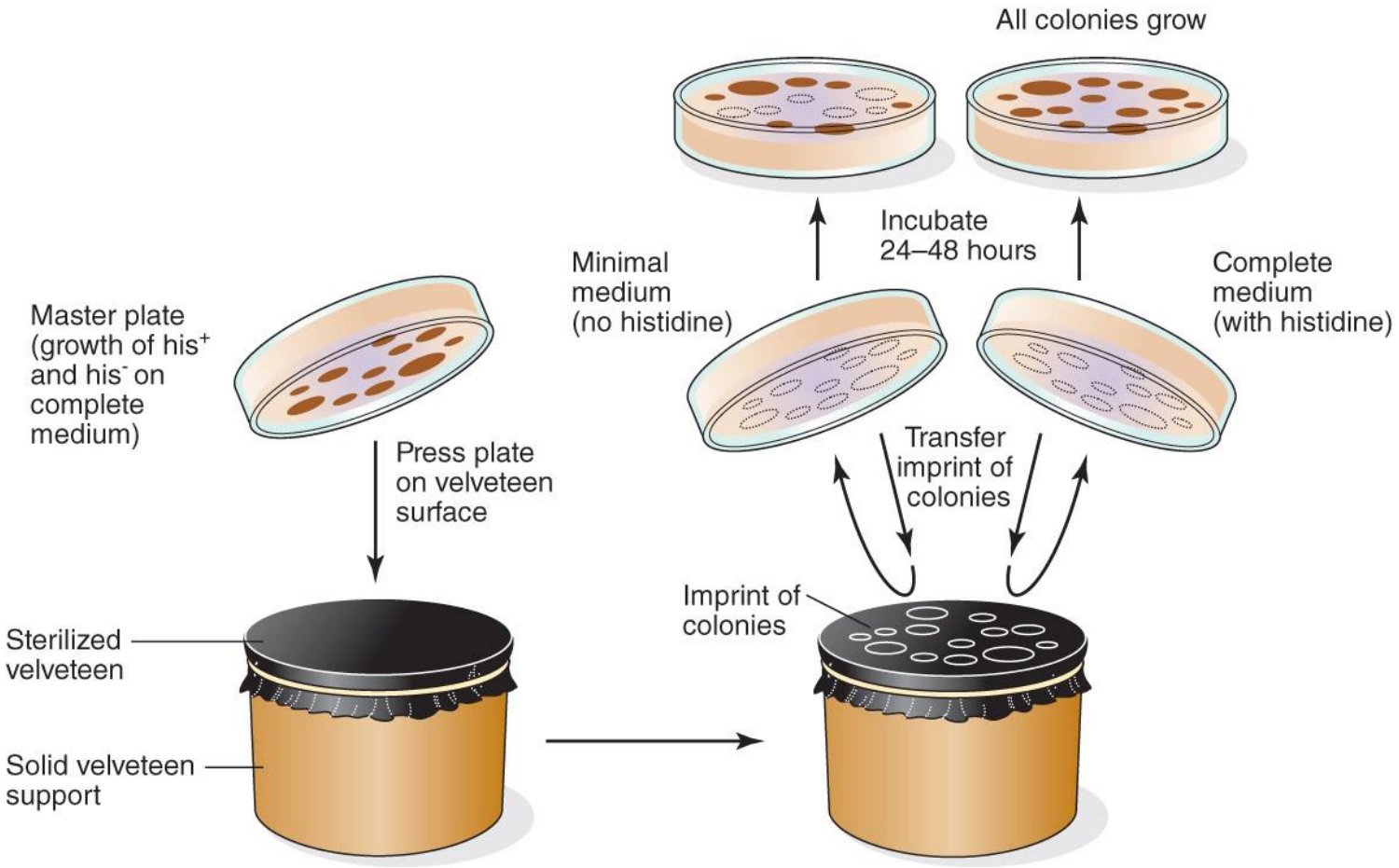
Techniques Exist for Identifying Mutants

- Plating Techniques Select for Specific Mutants or Characteristics
- The Ames test can identify potential mutagens
  - Mutagens cause revertants seen on medium lacking histidine


A A histidine-requiring strain (his⁻) of *Salmonella* is inoculated onto the surface of a plate of medium lacking histidine. Normally, the bacteria will not grow in the medium.
Well
B A well is cut into the agar and the suspected chemical mutagen is added to the well.
Diffusing chemical
C The suspected mutagen diffuses out from the well and comes in contact with the bacterial cells on the agar.
Incubate
Bacterial colonies
No growth
Growth
D If the chemical is not a mutagen, the bacterial cells remain unable to synthesize histidine and do not grow. Colonies fail to appear on the agar plate.
E If the chemical is a mutagen, some bacterial cells undergo a mutation so they can again synthesize histidine. Visible colonies soon appear on the agar plate.

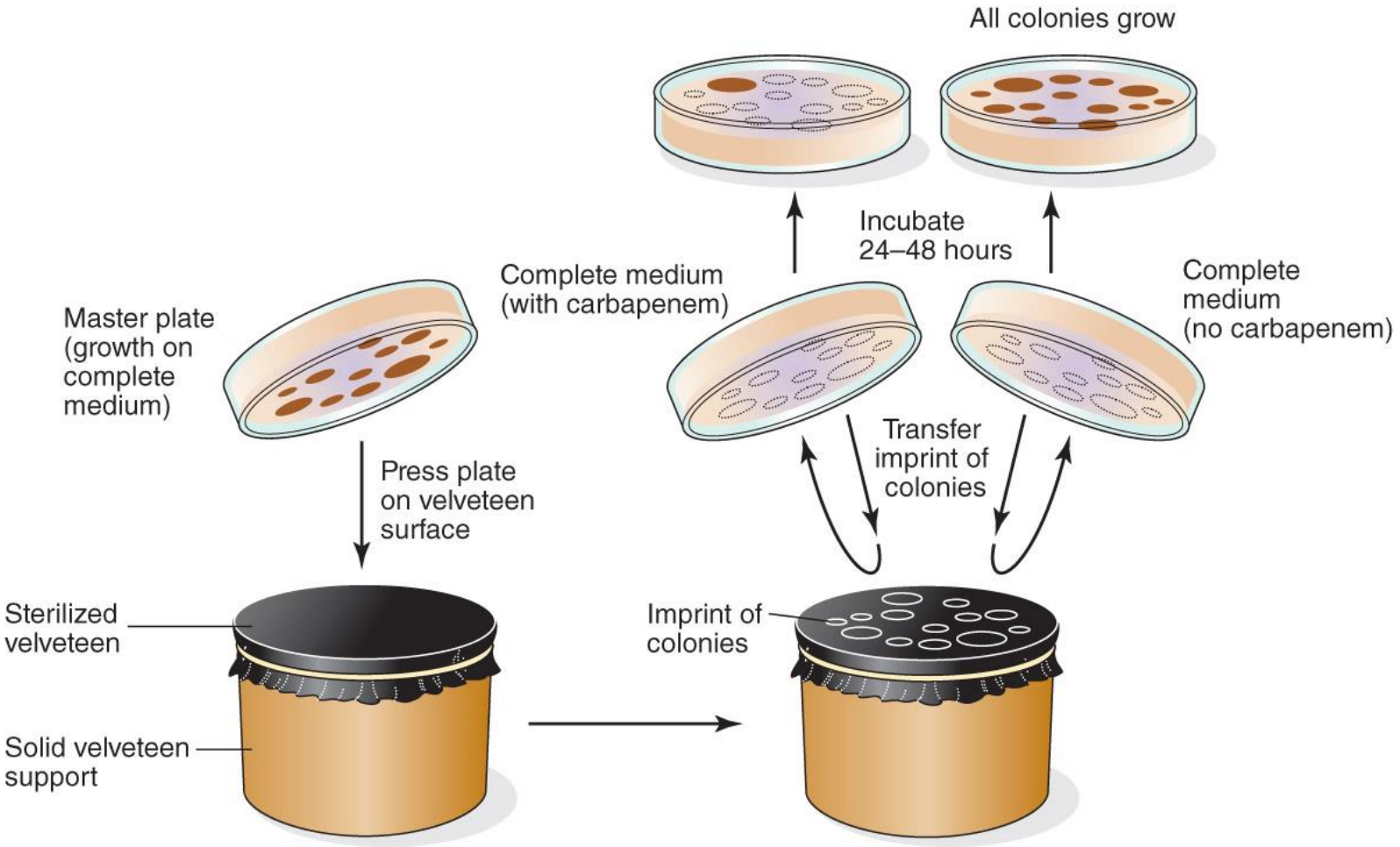
Using the Ames Test

- Negative selection is used to detect nutritional mutants that fail to grow on minimal media, when plated on complete nutritional medium.



Negative Selection Identifies Auxotrophs

- Positive selection of mutants shows resistant organisms growing on minimal medium.



Positive Selection of Mutants